



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Goddijn *et al.* **Docket No.:** U-011098-6
Serial No.: 08/779,460 **Examiner:** David Fox
Filed: 7 January 1997 **Group No.:** 1638
For: Enhanced Accumulation of Trehalose in Plants

TECH CENTER 1600/2900

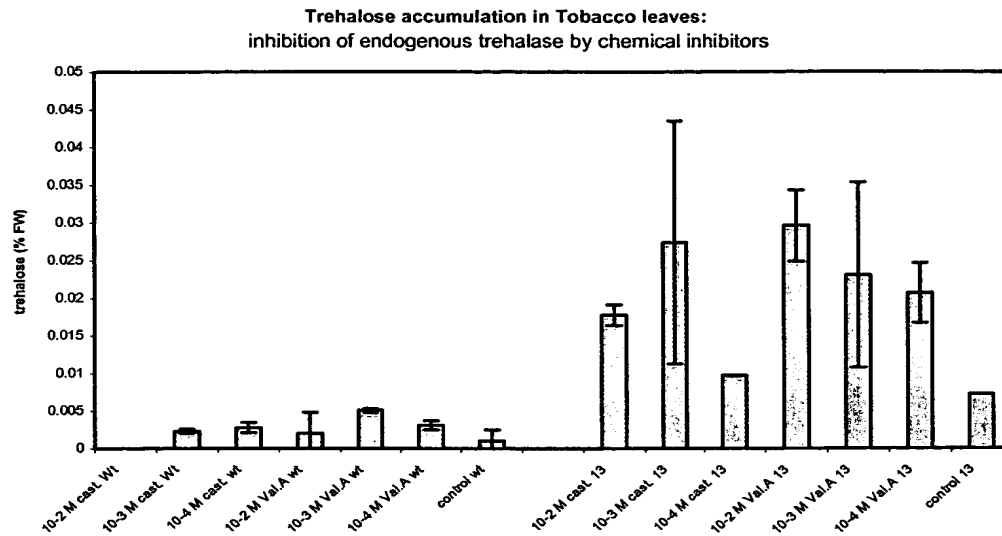
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DECLARATION

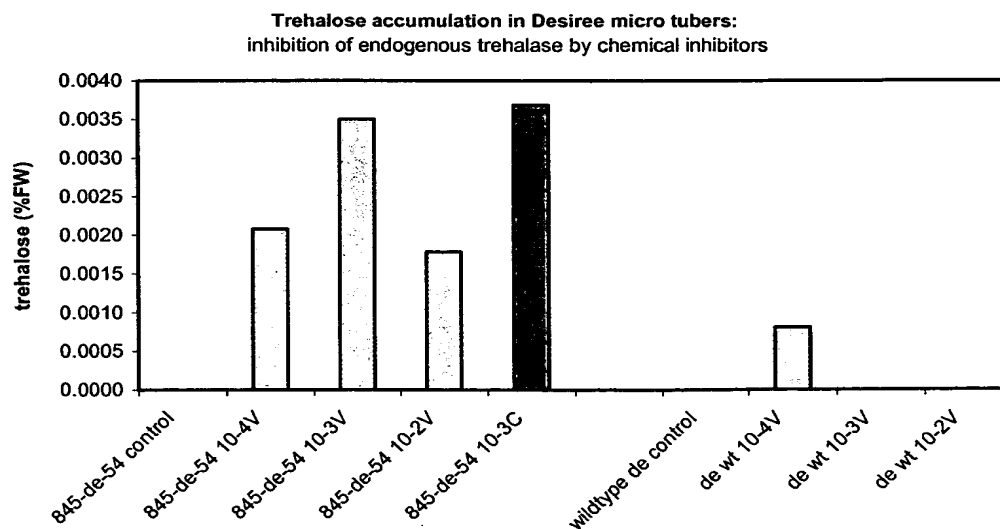
- (1) I, Anne Silene Ponstein, submit this Declaration in support of the above-identified patent application;
- (2) I am employed by Syngenta MOGEN (formerly Zeneca Mogen and previous to that Mogen International nv) in the capacity of Project Leader for a project relating to yield and my post office address is as follows: Einsteinweg 97, 2333 CB, Leiden, The Netherlands;
- (3) In support of the claims for the above application, appended below are the results of experiments carried out to show that trehalase inhibitors other than validamycin A are able to inhibit trehalase in a plant and thereby enhance accumulation of trehalose in plants;
- (4) Experiments were carried out in potato tuber material and in tobacco leaves to determine whether there was an enhanced accumulation of trehalose upon application of the trehalase inhibitor castanospermine from *Castanospermum australe* seeds. The experiments were conducted in the same way as indicated in the section headed "Experimental" in the specification for the above application. Example 1 of the specification details the experiment conducted for tobacco and Example 2 of the specification details the experiment conducted for potato micro-tubers. In both cases castanospermine was substituted for the inhibitor validamycin. Validamycin samples were run as a control.
- (5) The trehalose assay was conducted as follows. Trehalose was determined quantitatively by anion exchange chromatography with pulsed electrochemical detection. Extracts were prepared by adding 800ul 80% to 100-200 mg (fresh weight) frozen leaf material or freeze-dried tuber material. After homogenizing, the extracts were heated for 45 minutes at 75°C. The ethanol layer was dried in a vacuum centrifuge and the residue was taken up in 200ul nano pure water. Samples (25ul) were analyzed on a Dionex DX-300 liquid chromatograph equipped with a 4x250 mm Dionex 35391 carbopac PA-1 column and a 4x 50 mm Dionex 43096 carbopac PA-1 precolumn. Elution was with 100 mM NaOH at 1 ml/min. Sugars were detected with a Pulsed electrochemical detector (Dionex, PED-2) Commercially available trehalose (Sigma) was used as a standard.
- (6) The results of the experiment conducted in tobacco leaves are shown in Figure 1 below. As can be seen, trehalose accumulates in both transgenic plants (containing the construct denoted as '13', which contains TPS under the control of a plastocyanin promoter) and in wild-type plants upon administration of the trehalase inhibitor castanospermine (cast) or validamycin A (val A).

Figure 1



(7) The results of the experiments conducted in potato material are shown in Figure 2 below. A map of construct 845, which contains the TPS gene under the control of the patatin promoter, is shown in Figure 1 of the specification and was used in this experiment. As can be seen, trehalose accumulates in both transgenic and wild-type plants upon administration of castanospermine (C) or validamycin A (V).

Figure 2



(8) As can be seen from the above data, it is clear that trehalase inhibitors other than validamycin are able to inhibit endogenous trehalase and thereby enhance accumulation of trehalose in plants.

(9) The undersigned declares further that all statements of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the instant document and of application Serial No. 08/779,460 or any patents issuing thereon to which the instant document refers.

By:



Anne Silene Ponstein

Date:

12-12-2001